THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Effect of Low Temperature, Plant Age, and Photoperiod on the Photosynthesis of *Phalaenopsis* Hybrids

저온, 식물의 나이, 일장이 팔레놉시스의 광합성에 미치는 영향

BY

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AUGUST, 2017

MAJOR IN HORTICULTURAL SCIENCE AND BIOTECHNOLOGY
DEPARTMENT OF PLANT SCIENCE
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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ABSTRACT

This study was conducted to examine the low temperature effects under different vegetative growth stage and photoperiod effects on photosynthesis in *Phalaenopsis* hybrids. In Experiment 1, micropropagated clones of *Phalaenopsis* 'Hwasu 3551' (HS) and 'White Red Lip' (WR) were acclimated for 4 weeks. . The photoperiod was provided by 12 h with a photosynthetic photon flux density of 110 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the day (06:00~18:00). Plants were grown in a growth chamber at day/night temperatures of 28/26°C (high temperature, HT). Plants were transferred to 21/19^{o}C (low temperature, LT) after 0 (1-month-old), 2 (3-month-old), and 4 months (5-month-old) of cultivation at HT. In Experiment 2, for determining photoperiod effect, 1-month-old plants of *Phalaenopsis* 'Hwasu 355' were grown under short day (SD, 06:00~14:00), intermediate day (MD, 06:00~18:00), long day (LD, 06:00~22:00), and night interruption (NI, 06:00~18:00 + 22:00~02:00). Vegetative growth and diurnal CO_2 uptake rate of plants under each treatment were measured. Under all treatments, three *Phalaenopsis* hybrids showed typical CAM pathway, which is divided into Phase

I to IV by CO_2 exchange between leaf and air. When plants were transferred to LT, daily total net CO_2 uptake and growth rate decreased. In the plants transferred to LT at 3-month-old stage (after 2 months of cultivation at HT), CO_2 uptake rate during Phase III decreased to -1.36 and -0.60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in HS and WR, respectively, indicating CO_2 leakage. CO_2 leakage during Phase III induced the decrease of daytime total net CO_2 uptake and vegetative growth rate. In addition, when the plants were transferred to LT at 1-month-old stage (after 0 month of cultivation at HT), the number of new leaves significantly decreased. However, when 5-month-old plants were transferred to LT, decrease of the number of new leaves was not observed and only leaf length decreased. Under MD, leaf length, leaf width, and leaf area were significantly higher than LD and NI conditions. These results indicated that the effect of low temperature on photosynthesis and vegetative growth was small in 5-month-old plant, and vegetative growth was maintained. Also, vegetative growth rate under MD was higher than other photoperiods. As a result, maintaining high temperature (above 26^oC) with 12h/12h photoperiod is important during cultivation of young *Phalaenopsis* (1 and 3-month-old plant). This approach can helpful to schedule or strategize high quality *Phalaenopsis* cultivation and reduce damages from low temperature with energy savings.

Keywords: CAM plant, cost saving, CO_2 uptake, orchid, vegetative growth

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INTRODUCTION

Due to a variety of flower colors and long-lasting flowers, *Phalaenopsis* is one of the most important potted flowering orchids (Chen and Lin, 2012; Runkle et al., 2005). The global production of *Phalaenopsis* has been increasing over the years (Chen and Lin, 2012). For high quality *Phalaenopsis* production, vigorous growth during vegetative phase is important by maintaining relatively high temperature above 28^{o}C (Hew and Yong, 2004; Lopez et al., 2007; Pollet et al., 2011; Runkle et al., 2005). However, since production period is commonly over 1 year, maintaining relatively high temperature above 28^{o}C during cold season (as winter) is a big burden for growers at northern latitudes including Korea and Taiwan. If plants in vegetative growth stage are exposed to temperature below 26^{o}C in winter, the growth rate decreases and premature flowering which has low commercial value occurs (Lopez et al., 2007).

Temperature is a key factor affecting vegetative growth and flowering in *Phalaenopsis* (Gue and Lee, 2006; Ichihashi et al., 2006; Paradiso et al., 2012). Young plants of crassulacean acid metabolism (CAM) orchid including *Phalaenopsis* and *Doritaenopsis* (*Phalaenopsis* x *Doritis*) require a relatively high temperature (> 25^{o}C) for the increase of growth rate (Lee, 1991; Lin and Lee, 1988; Pollet et al., 2011; Wang, 2005). When the plants were exposed to relatively low temperature (< 25^{o}C) during vegetative growth stage, the increase rate of new leaf production and biomass decreased compared with those at relatively high temperature (> 25^{o}C) (Pollet et al., 2011; Sakanishi, 1980). In addition, photosynthetic ability decreased at temperature below 25/20^{o}C (day/night) (Gue

and Lee, 2008; Hew and Yong, 2004). Recently, to determine the optimal day/night temperature for vegetative growth and photosynthesis of CAM species, the diurnal net CO_2 uptake patterns of plants under different temperature regimes were compared at different growth stages (Goo and Lee, 2006; Jeon et al., 2006; Pimienta–Barrios, 2001; Pollet et al., 2011).

The response to photoperiod in *Phalaenopsis* hybrids showed that short day slightly induced flowering in smaller-flowered hybrids (Runkle et al., 2005). But, *Phalaenopsis* is basically non-photoperiodic under the inflorescence initiation temperature (< 26^{o}C). However, because CO_2 uptake of CAM plants is done by photosynthetic enzymes (PEPC and Rubisco) and their activity is affected by circadian rhythm, photoperiod influences the photosynthetic ability (Borland et al., 2000).

Photosynthesis is an important physiological process for growth of all green plants (Taiz and Zeiger, 2006). Photosynthetic ability of CAM plants can be estimated by measuring CO_2 uptake rate (Ichihashi et al., 2006; Jeon et al., 2006; Pimienta–Barrios, 2001; Pollet et al., 2011; Winter, 2002). In CAM plants, the diurnal net CO_2 uptake pattern is divided into four phases (Osmond et al., 1999; Borland et al., 2000). Phase I (nighttime) encompasses the main absorption of CO_2 through opened stomata and the storage of malate in vacuoles. The stored malate is decarboxylated and the released CO_2 is fixed in the Calvin cycle in phase III (daytime). In phase II and IV, CO_2 is absorbed through opened stomata and directly fixed by Calvin cycle (like C_3 photosynthesis) (Borland et al., 2000; Gue and Lee, 2006; Ichihashi et al., 2006; Luttge, 2004; Nelson et al., 2005; Taiz and Zeiger, 2006). Because each phase has an optimal temperature for CO_2

absorption, proper day/night temperature regimes for increasing daily net CO_2 uptake are important (Gue and Lee, 2006; Ichihashi et al., 2006; Kano and Naitoh, 2001; Nelson et al., 2005; Ota et al., 1991; Ota et al., 2001; Taize and Zeiger, 2006).

In *Phalaenopsis* showing a typical CAM pathway, 30/25^{o}C (day/night) temperature regime is usually used for vigorous vegetative growth in Taiwan (Gue and Lee, 2006; Hung, 1998; Lee, 1988). When plants were exposed to a relatively low temperature below 25/20^{o}C during vegetative growth phase, total net CO_2 uptake decreased and vegetative growth was delayed compared with those grown at a relatively high temperature.

Although there are many previous researches which reported temperature effects on photosynthesis or vegetative growth in *Phalaenopsis*, only a few reports about low temperature effects on both photosynthesis and vegetative growth at different growth stages have been published. The objective of this study was to determine low temperature effects at different vegetative growth stages and photoperiod effects on photosynthesis and vegetative growth in two *Phalaenopsis* hybrids.

LITERATURE REVIEW

Photosynthesis of CAM Plants

In CAM plants including cacti, *Hoya*, and *Phalaenopsis*, the CO_2 fixation is mainly progressed in the dark period. CO_2 uptake pattern showed a diurnal fluctuation, and it is divided into four phases (Hew and Yong, 2004; Taize and Zeiger, 2006)

Phase I: Nocturnal net CO_2 uptake is mediated by phosphoenolpyruvate carboxylase (PEPC). Absorbed CO_2 through opened stomata is incorporated via carboxylation of phosphoenolpyruvate (derived from starch or glucan) to oxaloacetate by PEPC in the cytosol, and then reduced to malate. The malate is stored in the vacuoles of leaf cell (Hew and Yong, 2004; Taize and Zeiger, 2006) and then it is used during Phase III.

Phase II: At the beginning of daytime, CO_2 uptake occurs by PEPC through opened stomata. CO_2 uptake during Phase II is influenced by PEPC phosphorylation, which is regulated by malate content during the first 2~3 hours of the daytime in *Clucia minor* and *Kalanchoe daigremontiana* (Borland and Griffiths, 1997; Fischer and Kluge, 1984). The enhanced PEPC phosphorylation resulted in the increase of CO_2 uptake during Phase II (Borland and Griffiths, 1997).

Phase III: In the midday, there is no or little CO_2 exchange by closed stomata because of preventing water loss. For CO_2 assimilation by Calvin cycle, malic acid is consumed. Malate stored in vacuole during Phase I is transferred to cytosol and decarboxylation of malate is usually achieved by NADP-malic enzyme

(NADP-ME) (Drincovich et al., 2001). Because of the closed stomata, the released CO_2 cannot efflux from the leaf tissue to air and instead is fixed by the Rubisco and converted to carbohydrate through the Calvin cycle (Taize and Zeiger, 2006).

Phase IV: In the late afternoon, very low partial pressure of CO_2 (pCO_2) and high Rubisco activity induce Rubisco mediated CO_2 uptake (Borland et al., 2000). In this phase, because of high photorespiration rates (Maxwell et al., 1997), photoprotective process as non-photochemical quenching (NPQ) of chlorophyll fluorescence increases.

CAM plants such as cacti, *Hoya*, *Clusia*, *Cattleya*, and *Phalaenopsis* mainly absorb CO_2 in the nighttime (unlikely C_3 photosynthesis) and photosynthesis pattern is divided into four phases. According to Taiz and Zeiger (2006), Phase I encompasses the nocturnal net CO_2 uptake by phosphoenolpyruvate carboxylase (PEPC), synthesizing malate and the storage of malate in vacuoles. The stored malate is decarboxylated by NADP-malic enzyme (NADP–ME) and released CO_2 is fixed by Calvin-cycle during Phase III (Borland et al., 2000; Borland et al., 2011; Edwards et al., 1992; Taiz and Zeiger, 2006). Because these phases are progressed by several enzymes (PEPc, malate dehydrogenase, and NADP--ME), there is the optimal temperature for CO_2 uptake and CO_2 assimilation. For the maximal CO_2 uptake rate of CAM photosynthesis, plants require relatively low temperature in the nighttime (Phase I) and relatively high temperature in the daytime (Phase III) (Bradon, 1967).

Factors Affecting Photosynthesis of CAM Plants

***Temperature*:** Photosynthetic ability of CAM plants is influenced by day/night

temperatures (Hew and Yong, 2004). Because photosynthetic pathway of CAM plants is progressed by PEPC (CO_2 absorption in Phase I), Rubisco (Calvin cycle in Phase III), and NADP-ME (malate decarboxylation in Phase III), the temperature management for optimal photosynthesis is needed (Taize and Zeiger, 2006). Nocturnal and daytime CO_2 uptake rate of *Phalaenopsis* reaches the peak at 20°C and 30°C, respectively, and decreases at higher or lower temperatures than these temperatures (Guo and Lee, 2006; Hew and Yong, 2004).

Light: High irradiance enhanced malate decarboxylation and CO_2 assimilation through Calvin cycle during Phase III and PEPC activity during Phase I (Luttge, 2008). Thus, in the daytime, increase of light intensity enhanced CO_2 absorption during Phase I (Hew and Yong, 2004). However, each plant has light saturation point for photosynthesis, and there is an optimal intensity range for photosynthesis (180 to 215 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Phalaenopsis amabilis*) (Chen and Lin, 2012). Also, because CO_2 uptake pattern is progressed by several enzymes (PEPC, NADP-ME and etc.), it is directly connected to the circadian rhythm of their activity (Nimmo et al., 1984). In *Phalaenopsis* cultivation, 12/12h (day/night) photoperiod is recommended for maximum CO_2 uptake (Guo and Lee, 2006)

Age: In very young *Dendrobium* and *Phalaenopsis* plantlet with thin leaves (protocorm), malate and PEPC activity showed no diurnal fluctuation (Hew and Yong, 2004; Ping et al., 2010). While plants age, leaves thicken and begin to show fluctuation of PEPC activity and change of CAM capacity following the organ maturity (Ping et al., 2010). For *Aranda*, *Arachnis*, and *Phalaenopsis*, CAM capacity reached a peak when leaves have just attained maturation, and photosynthetic ability decreased significantly with aging (Hew and Yong, 2004;

Ota et al., 1991).

MATERIALS AND METHODS

Experiment 1. Effect of Low Temperature and Plant Age on the Photosynthesis of *Phalaenopsis* 'Hwasu 3551' and 'White Red Lip'

Plant Materials and Growth Conditions

Clones of micropropagated *Phalaenopsis* hybrid 'Hwasu 3551' (HS) and 'White Red Lip' (WR) with two fully-developed leaves were purchased from Sang Mi Orchids, LLC. (Taean, Korea) and transplanted in 3.81 cm pot filled with 100% sphagnum moss (Chilean dried sphagnum moss, Lonquen Ltd., Puerto Montt, Chile) on 20 Jun. 2013. All plants were acclimated in a growth chamber (HB-301MP, Hanbaek Scientific Co., Bucheon, Korea) maintained at a day/night temperatures of 28/26^{o}C and relative humidity (RH) of 70-80%for 4 weeks. During acclimation, 12h photoperiod was provided photosynthetic photon flux densities (PPFD) of 30 (first 2 weeks) and 60 (last 2 weeks) $\mu mol \cdot m^{-2} \cdot s^{-1}$ (필요할 경우는 광도센서와 모델명을 별도 문장으로 기술).

The plants were fertigated once a week by overhead irrigation with water soluble fertilizer (EC 1.0 $mS \cdot cm^{-1}$; Technigro 20N-9P-20K Plus fertilizer, Sun-Gro Horticulture, WA, USA). When experiment began (after acclimation), leaf length of 'Hwasu 3551' and 'White Red Lip' was 5.05 cm and 4.50 cm, respectively.

Temperature Treatments

After acclimation for 4 weeks (plant age was 1-month-old), plants with 2-3 fully-developed leaves were transferred to another growth chamber at 28/26^{o}C(relatively high temperature, HT). In this chamber, RH was 60-70% and 12 h photoperiod (06:00-18:00) was provided by 110 ± 10 μmol·m^{-2}·s^{-1} PPFD with high pressure metal halide lamps (MH250W, Hanyoung Electric Co., Gwangju, Korea) and three-wave cool white fluorescent lamps (EFTR20EX-D, Hangzhou Li-Tech Electric Co. Ltd., Hangzhou, China).

To determine the effects of low temperature at different vegetative growth stages, 15 plants grown at HT in each hybrid were transferred to 21/19^{o}C (relatively low temperature, LT) growth chamber after 0, 2, and 4 months (1, 3 and 5-month-old, respectively) of cultivation at HT. LT was applied for 3 months, and other environmental conditions were the same as those of growth chamber at HT.

Data Collection

Plant growth was measured as the number of new leaves, leaf length, leaf width and thickness of the uppermost fully expanded leaf every month for each plant. The growth rate during 3 months period under two temperature regimes at LT and HT was calculated and compared each other.

Net CO_2 uptake pattern was measured by a portable photosynthesis system with an infrared gas analyzer (Li 6400, Li-Cor Co., Inc., Lincoln, NE, USA). The uppermost fully expanded leaves were clamped onto a top clear leaf chamber (3 m x 2 m). In leaf chamber, the temperature was the same as the growth chamber, and CO_2 concentration and RH were 600 μmol·m^{-2}·s^{-1} and 40-60%, respectively.

Diurnal changes in net CO_2 uptake pattern was recorded every 2 h for 24 h from three randomly selected plants. For measuring this pattern, we used the plants maintained at HT followed by LT for 3 months (LT treatment started after 0, 2, and 4 months of cultivation at HT). Nighttime, daytime, and daily total net CO_2 uptakes were calculated by using 'Area below curve' function of SigmaPlot software ver. 10.0 (Systat Software, Inc., Chicago, IL, USA).

Statistical Analysis

All the data were analyzed by the SAS system for windows ver. 9.3 (SAS Inst. Inc., Cary, NC, USA). The means of each treatment were assessed using T-test at $P < 0.05$. Regression and graph module analysis were performed using SigmaPlot software ver. 10.0 (Systat Software, Inc., Chicago, IL, USA).

Experiment 2. Effect of Photoperiod on the Photosynthesis of Phalaenopsis 'Hwasu 355'

Plant Materials and Growth Conditions

Clones of micropropagated *Phalaenopsis* hybrid 'Hwasu 355' with three fully-developed leaves were purchased from Sang Mi Orchids, LLC. (Taean, Korea) and transplanted in 3.81 cm pot filled with 100% sphagnum moss (Chilean dried sphagnum moss, Lonquen Ltd., Puerto Montt, Chile) on 29 Dec. 2013. All plants acclimated in a growth module at day/night temperatures fo 28/26^{o}C and RH of 60-80% for 4 weeks. During acclimation, 12h photoperiod was provided by a PPFD of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using ??? (HPI-T Plus Philips, 400W lamps, Philips,

Eindhoven, Netherlands).

The fertigation strategy was same to Exp. 1. When experiment began (after acclimation), number of leaves, leaf length, and leaf width were 3.9, 14.1, and 3.2 cm, respectively.

Photoperiod Treatments

After acclimation for 4 weeks, 30 plants with 3-4 fully-developed leaves each treatment were transferred to another four independent growth module at 28/26^{o}C and 60-70 % RH.

To determine the effects of photoperiod on vegetative growth and photosynthesis of *Phalaenopsis* 'Hwasu 355', day lengths of 8 (06:00~14:00, SD), 12 (06:00~18:00, MD), 16 (06:00~22:00, LD), and 12+4 h (06:00~18:00 + 22:00~02:00, NI) at a PPFD of 110 ± 10 μmol·m^{-2}·s^{-1} were provided to each growth module.

Data Collection

Plant growth was measured as the number of leaves, leaf length, leaf width, and thickness of the uppermost fully expanded leaf every month for each plant. Diurnal net CO_2 uptake pattern was measured at 4 months after treatment (25 Apr., 2014) by a portable photosynthesis system with an infrared gas analyzer (Li 6400, Li-Cor Co., Inc., Lincoln, NE, USA). The leaf position and method for measurement was same to Exp. 1.

Statistical Analysis

All the data were analyzed by the SAS system for windows ver. 9.3 (SAS Inst. Inc., Cary, NC, USA). The means of each treatment were assessed using Duncan's honestly significant different test at $P < 0.05$. Regression and graph module analysis were performed using SigmaPlot software ver. 10.0 (Systat Software, Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Experiment 1. Effect of Low Temperature and Plant Age on the Photosynthesis of Phalaenopsis 'Hwasu 3551' and 'White Red Lip'

Effects of Low Temperature on Vegetative Growth

***Phalaenopsis* 'Hwasu 3551':** Over all, when plants grown at HT were transferred to LT, the number of new leaves, leaf length, and leaf width decreased. However, leaf thickness increased (Fig. 1D). In the number of new leaves and leaf width, there was no significant decrease when plants were transferred to LT at 5-month-old stage (after 4 months of cultivation at HT), but 1 and 3-month-old plants showed significant decrease (Figs. 1A, C). Leaf length showed significant decrease in all plant age when transferred to LT (Figs. 1B). In contrast, leaf thickness of 1, 3, and 5-month-old plants significantly increased in plants transferred to LT (Fig. 1D).

***Phalaenopsis* 'White Red Lip':** Changes of vegetative growth rate under LT was similar to those of *Phalaenopsis* 'Hwasu 3551'. The number of new leaves did not significantly decrease in only 5-month-old plant (Fig. 2A). For leaf length, there was significant decrease in 1, 3, and 5-month-old plants (Fig. 2B). Leaf width decreased in 1 and 5-month-old and slightly increased in 3-month-old plants under LT condition, but there was no significant difference (Fig. 2C). In leaf thickness, there was significant increase in 3 and 5-month-old plants (Fig.

2D).

Because new shoot and leaf production at the initial stage is essential for vigorous vegetative growth in micropropagated plants such as *Phalaenopsis*, *Cymbidium*, *Vanda*, and *Dendrobium* (Hew and Yong, 2004), the decrease of new leaf production under relatively low temperature conditions is not suitable for vigorous vegetative growth. In contract, low temperature effect was small when plants were transferred at 5 months or older (after more than 4 months of cultivation at HT).

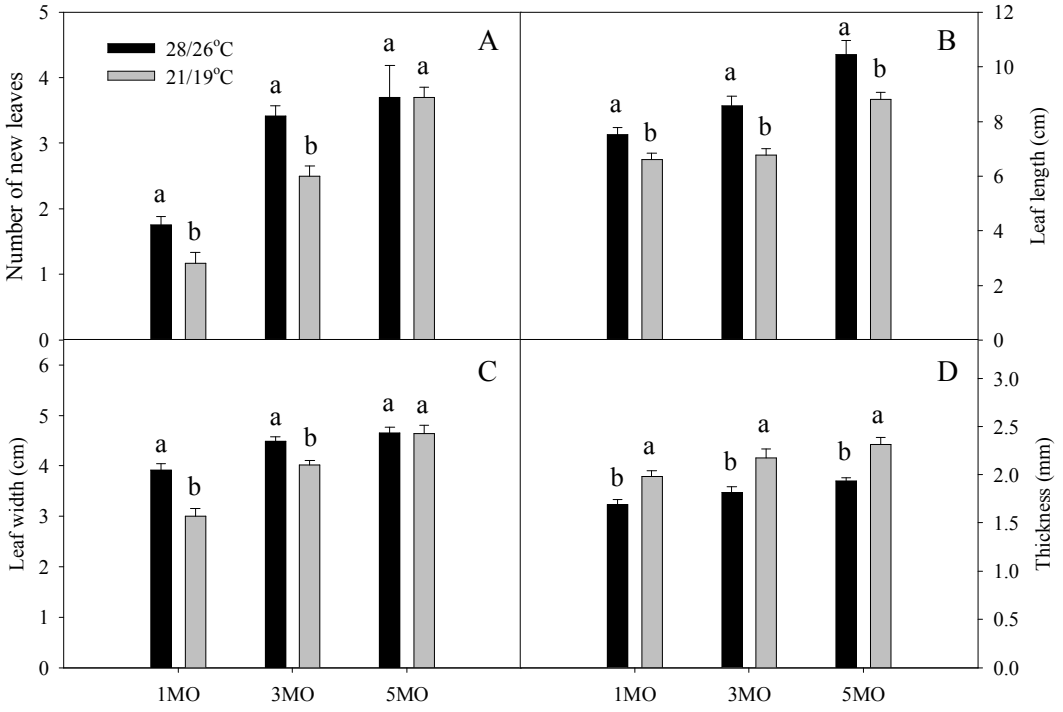

Fig. 1. Vegetative growth in *Phalaenopsis* 'Hwasu 3551' at day/night temperatures of 28/26°C and 21/19°C. After 0 (1-month-old, 1MO), 2 (3-month-old, 3MO), and 4 (5-month-old, 5MO) months of cultivation at 28/26°C, plants were transferred to 21/19°C growth chamber and cultivated for 3 months. Vertical bars represent SE (n = 9). Mean separation for each temperature by T–test at 5% level.

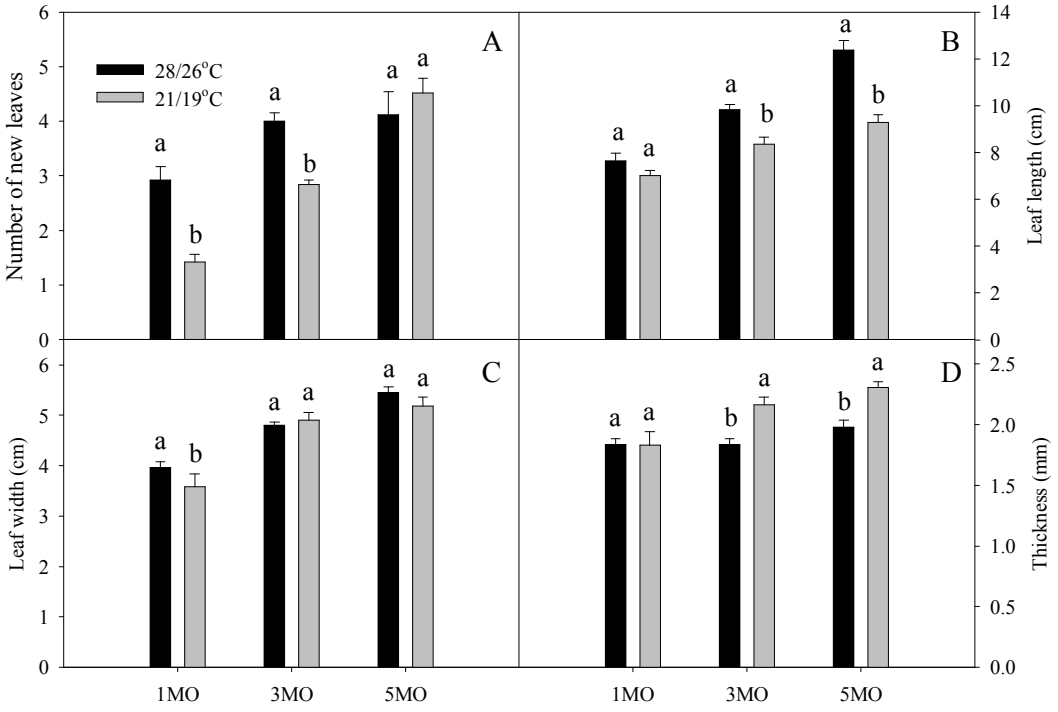

Fig. 2. Vegetative growth in *Phalaenopsis* 'White Red Lip' at day/night temperatures of 28/26°C and 21/19°C. After 0 (1-month-old, 1MO), 2 (3-month-old, 3MO), and 4 (5-month-old, 5MO) months of cultivation at 28/26°C, plants were transferred to 21/19°C growth chamber and cultivated for 3 months. Vertical bars represent SE (n = 9). Mean separation for each temperature by T-test at 5% level.

Pattern of Net CO_2 Uptake

Diurnal net CO_2 uptake pattern was affected by several factors, which might be attributable to plant age and temperature. *Phalaenopsis* 'Hwasu 3551' and 'White Red Lip' showed typical CAM pathway during 24 hour measurements (Fig. 3). Major CO_2 uptake occurred during the nighttime (Phase I). During the dark period, the nocturnal net CO_2 uptake rate increased and reached its peak, then decreased rapidly during early part of the day (Phase II). During midday (Phase III), CO_2 uptake was not observed (Figs. 3 A, B, E and F). However, in 3-month-old plants, CO_2 leakage occurred when transferred to LT (Figs. 3 C and D). The CO_2 uptake rate increased again during the late afternoon (Phase IV).

The diurnal net CO_2 uptake pattern showed typical CAM photosynthesis and fluctuated for three plant ages (1, 3 and 5-month-old) and temperature regime (HT and LT). When plants were transferred to LT in 1-month-old plants, the nocturnal CO_2 uptake rates of two hybrids (Phase I) decreased compared with those maintained at HT, and CO_2 uptake rate during phase III was almost the same in two temperature regimes (Figs. 3A and B). In plants transferred to LT at 3-month-old stage (after 2 months cultivation at HT), CO_2 uptake rate during phase III decreased to -1.36 and -0.60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in both cultivars and showed CO_2 leakage, respectively (Figs. 3C and D). CO_2 loss by leakage during Phase III (and entire phases) reduced photosynthetic efficiency (CO_2 assimilation) of CAM plants (Borland et al., 2000; Maxwell et al., 1997), and the leakage rate is largely controlled by stomatal conductance (Evans and Loreto 2000). Because stomatal conductance during Phase III in both HS and WR transferred to LT were higher than those maintained at HT (data not shown), it was assumed that CO_2 leakage

occurred and daytime total net CO_2 uptake decreased. These results are similar to the results of previous studies by Friemart et al. (1986) with *Kalanchoe tubiflora* Hamet and *Sedum morganianum* E. Waith and by Maxwell et al. (1997) with *Kalanchoe daigremontiana*. On the other hand, nocturnal CO_2 uptake rate increased compared with those maintained at HT. When plants were transferred to LT at 5-month-old age (after 4 months of cultivation at HT), the nocturnal CO_2 uptake rates of two hybrids (Phase I) slightly decreased compared with those maintained at HT, but overall changes of CO_2 uptake rates during 24 hour period in plants transferred to LT and plants maintained at HT showed similar pattern (Figs. 3E and F).

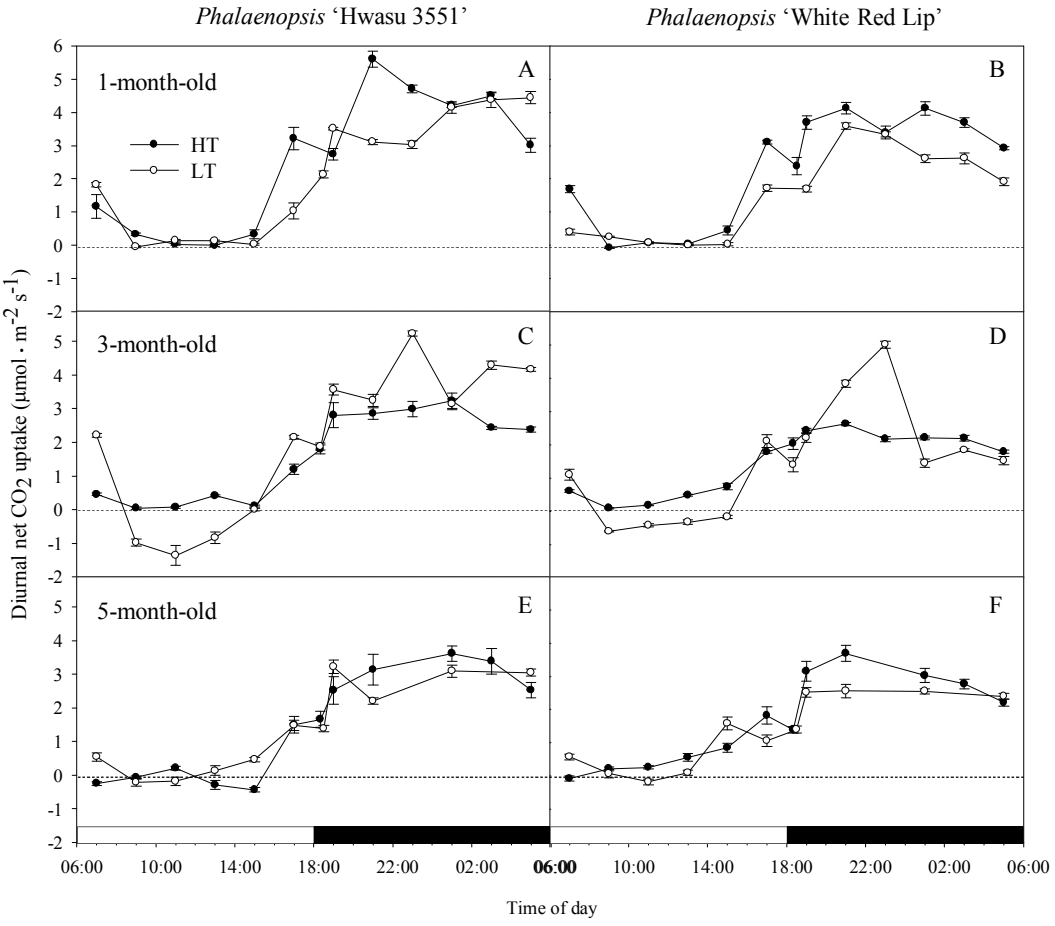

Fig. 3. Diurnal net CO_2 uptake patterns of plants maintained at 28/26°C (HT, closed circle) or transferred to 21/19°C (LT, open circle) for 3 months at three vegetative growth stages: 1 (A and B), 3 (C and D) and 5 (E and F) -month-old *Phalaenopsis* 'Hwasu 3551' (A, C and E) and 'White Red Lip' (B, D and F). Vertical bars represent SE (n = 3). □ = daytime and ■ = nighttime.

Total Net CO_2 Uptake for 24h

Nighttime, daytime, and daily total net CO_2 uptakes were calculated from diurnal net CO_2 uptake pattern in Fig. 3 (Fig. 4). When plants were transferred to LT condition at 1-month-old stage (after 0 months of cultivation at HT), the nocturnal, daytime, and daily total net CO_2 uptake decreased compared with those maintained at HT (Figs. 4A and B). In plants transferred to LT condition at 3-month-old stage (after 2 months of cultivation at HT), the total nocturnal net CO_2 uptake was higher than those at HT in both cultivars (Figs. 4C and D). However, because the daytime total net CO_2 uptake significantly decreased in plants transferred to LT, daily total net CO_2 uptake did not significantly increase (Figs. 4 C and D).

There was no significant difference in nighttime, daytime, and daily total net CO_2 uptake between transferring to LT at 5-month-old stage (after 4 months cultivation at HT) and maintaining at HT (Figs. 4E and F). Bradon (1967) reported that the optimal temperature for PEPC, malate dehydrogenase, and the decarboxylation enzyme (NADP-ME) was 35^{o}C, 35^{o}C, and above 53^{o}C, respectively, by in vitro study. However, due to complex temperature interactions in photosynthetic pathway and respiration, total daily net CO_2 uptake was the highest at 30/20^{o}C (Israel and Nobel, 1995).

Temperature below 30/25^{o}C induced the decrease of total net CO_2 uptake and prolonged vegetative growth (Gue and Lee, 2006). *Phalaenopsis amabilis* showed the maximum daily and nocturnal total net CO_2 uptake at 32/28 ^{o}C or 29/25^{o}C, and relatively low temperature (25/20 ^{o}C and 21/16^{o}C) reduced total daily and nocturnal net CO_2 uptake. These results about net CO_2 uptake for 24 hour were

consistent with their vegetative growth. *Phalaenopsis* hybrids showed the highest production rate of new leaves at 30/25^{o}C and the vegetative growth rate was decreased at 20/15^{o}C (Lee and Lin, 1984, 1987; Lin, 1994; Lin and Lee, 1988; Wang, 2005).

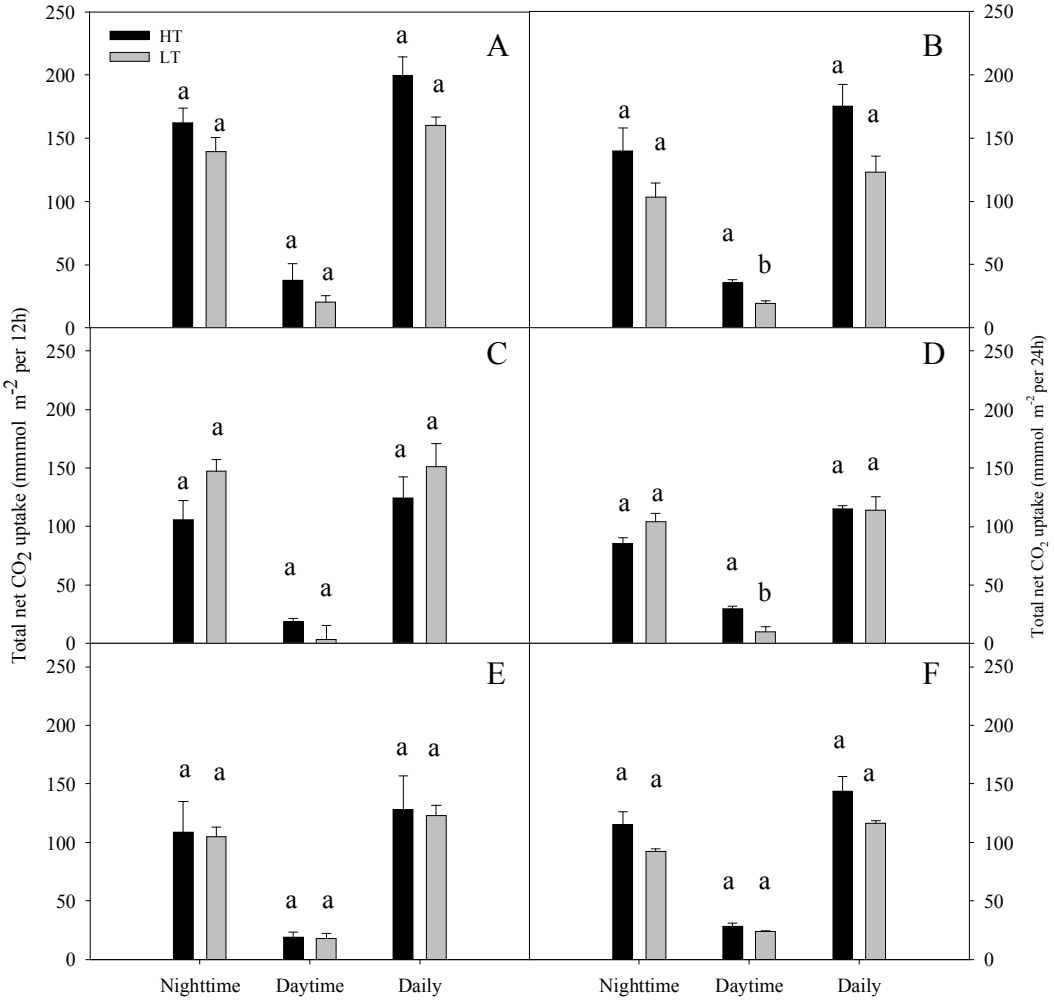

Fig. 4. Nighttime, daytime, and daily total net CO_2 uptake of plants maintained at 28/26°C (HT) or transferred to 21/19°C (LT) for 3 months at three vegetative growth stages [1 (A and B), 3 (C and D) and 5 (E and F)-month-old] in *Phalaenopsis* 'Hwasu 3551' (A, C, and E) and 'White Red Lip' (B, D, and F). Data were obtained by calculating net CO_2 uptake curves in Fig. 3. Vertical bars represent SE (n = 3). Mean separation within each stage by T-test at 5% level.

Experiment 2. Effect of Photoperiod on Photosynthesis of Phalaenopsis 'Hwasu 355'

Effects of Photoperiod on Vegetative Growth

Plants showed no significant difference in the number of leaves and leaf thickness among all treatments (Table 1). Under MD, leaf length, leaf width, and leaf area were higher than those under SD, but there was no significant difference. Plans grown under MD had the significantly longer leaf length (8.53 cm) compared with those under LD and NI by 111.9% and 111.4%, respectively. Leaf width and leaf area were significantly longer and larger in plants under MD compared with those under LD and NI.

Patterns of Net CO_2 Uptake

Phalaenopsis 'Hwasu 355' showed typical CAM pathway with four phases during 24 hour measurements (Fig. 5) under SD, MD, and LD condition. However, under NI, rapid decrease of CO_2 uptake rate from 2.82 to 0.19 $\mu mol \cdot m^{-2} \cdot s^{-1}$ occurred during NI period (22:00–02:00). Plants showed different maximal CO_2 uptake rate during the daytime as 0.72, 1.94, 3.3, and 4.53 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under SD, MD, LD, and NI, respectively.

Total Net CO_2 Uptake for 24h

Nighttime, daytime, and daily total net CO_2 uptakes were calculated from diurnal net CO_2 uptake pattern from Fig. 5. Under SD and MD, nighttime total net CO_2 uptake showed no significant difference between two treatments, but they

were significantly higher than those under LD and NI. In contrast, daytime total net CO_2 uptake under LD and NI were significantly higher than those under SD and MD. Daily total net CO_2 uptake was the highest under LD, while the lowest under SD among treatments.

These results could be attributed to CO_2 uptake pattern and photosynthetic ability during the daytime. In CAM photosynthesis, CO_2 fixed through Calvin cycle was provided by malate decarboxylation (Taize and Zeiger, 2006). When malate was consumed in Phase III, intercellular CO_2 concentration (Ci) and partial pressure of CO_2 (pCO_2) in leaf tissue decreased. Low pCO_2 and activated Rubisco induced CO_2 uptake during phase IV (Borland et al., 2000). However, because photorespiration rate was high in phase IV (Maxwell et al., 19997, 1998), photoprotective process was manifested as an increase of non-photochemical quenching (NPQ) of chlorophyll florescence (Horton et al., 1996). These results suggest that increase of CO_2 absorption during Phase IV was not closely related to photosynthetic capacity. Similarly, Guo and Lee (2012) reported that 12/12h (day/night) photoperiod was suitable to photosynthesis of *Phalaenopsis amabilis*.

Table 1. Effects of photoperiod on the number of leaves, leaf length, leaf width, leaf area, and thickness of *Phalaenopsis* 'Hwasu 355' at 4 months after treatment.

	Number of leaves	Leaf length[z] (cm)	Leaf width (cm)	Leaf area (cm^2)	Thickness (mm)
SD	6.0 a[y]	8.45 a	4.20 a	35.58 a	1.68 a
MD	6.3 a	8.53 a	4.36 a	37.19 a	1.72 a
LD	6.3 a	7.62 b	3.86 b	29.52 b	1.75 a
NI	5.9 a	7.66 b	3.62 c	27.78 b	1.70 a

[z]Length of the uppermost fully expanded leaf.

[y]Mean separation within columns by Duncan's honestly significant difference test at $p < 0.05$.

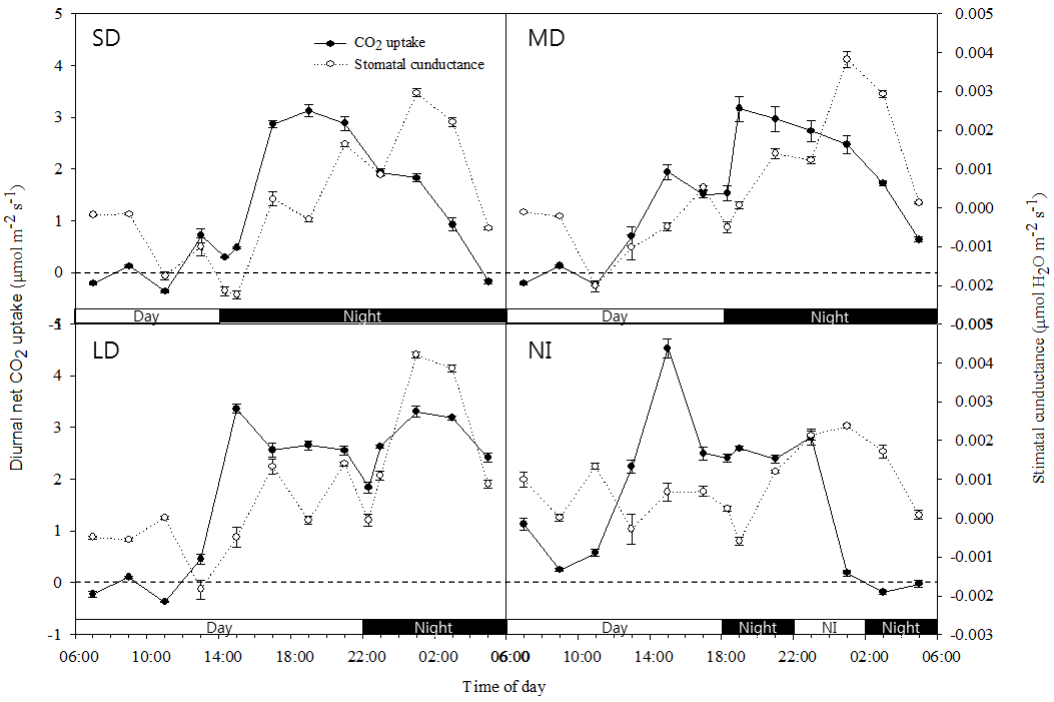

Fig. 5. Diurnal net CO_2 uptake and stomatal conductance patterns of *Phalaenopsis* 'Hwasu 355' under short day (SD), intermediate day (MD), long day (LD), and night interruption (NI) at 4 months after treatment. Vertical bars represent SE (n = 3). □ = daytime and ■ = nighttime

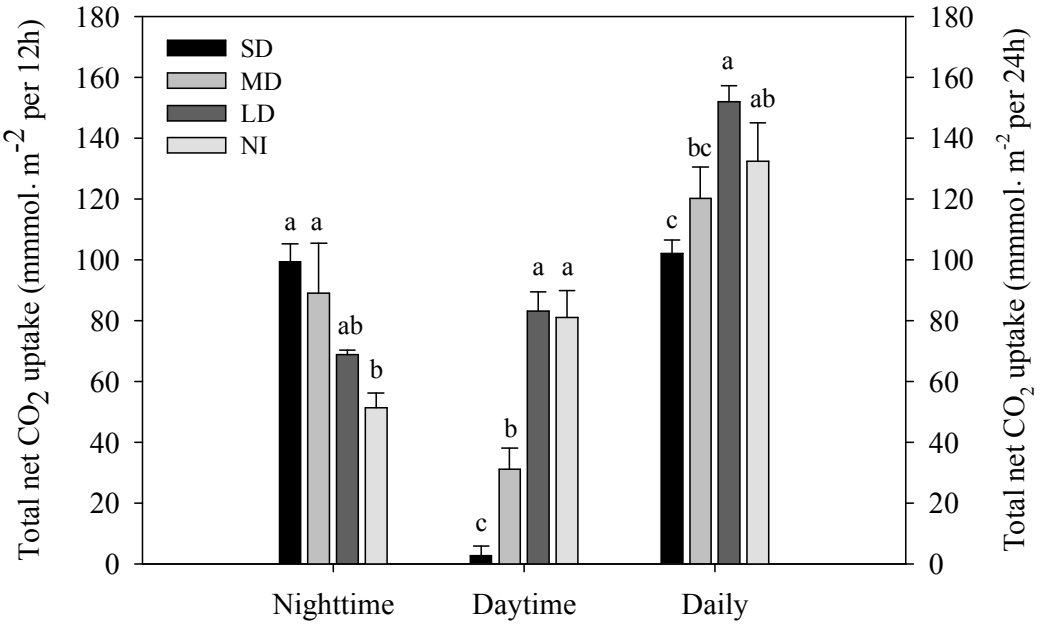

Fig. 6. Nighttime, daytime, and daily total net CO_2 uptake of *Phalaenopsis* 'Hwasu 355' under short day (SD), intermediate day (MD), long day (LD), and night interruption (NI) at 4 months after treatment. Data were obtained by calculating net CO_2 uptake curves in Fig. 5. Vertical bars represent SE (n = 3). Mean separation for each treatment by Duncan's honestly significant difference test at $p < 0.05$.

In conclusion, our study demonstrated that relatively low temperature at 21/19^{o}C induced the decrease of photosynthetic ability and vegetative growth rate compared with those of relatively high temperature at 28/26^{o}C. These results were affected by plant age, and low temperature effect on photosynthesis and vegetative growth of HS and WR was small when plants were transferred to LT at 5-month-old (after 4 months of cultivation at HT). Even though conversion timing from vegetative stage to reproductive stage is different by cultivars (An et al., 2013; Paradiso et al., 2012), usually, young plants before 6~7 month old did not respond to flower at relatively low temperature below 26^{o}C. Thus, it is reasonable to suggest that cost-saving strategy of reducing greenhouse heating and maintaining vegetative growth is possible in 5-month-old stage during vegetative growth of *Phalaenopsis*.

Because we did not investigate C_4 acid (malate) and enzymes (PEPC, NADP-ME, and dehydrogenase), the study about metabolism of absorbed CO_2 and accumulation of dry materials are needed for further mechanistic study. This information will be useful for improving strategy in *Phalaenopsis* cultivation.

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ABSTRACT IN KOREAN

본 실험은 호접란의 광합성에 있어서 저온이 각 생육단계별로 끼치는 영향과 일장 조건의 영향을 알아보고자 수행하였다. 첫 번째 실험을 위해 조직배양을 통해 번식된 호접란 'Hwasu3551' 과 'White Red Lip' 두 품종의 유묘들을 4주간 순화한 후(1개월 묘), 28/26°C (고온, HT)의 식물생육상에서 재배하였다. HT에서 0(1개월 묘), 2(3개월 묘), 4 개월(5개월 묘)간 재배한 식물들을 21/19°C(저온, LT)의 식물생육상으로 옮겨 3개월간 저온처리 하였고, 각 식물생육상의 일장은 12시간(06시–18시), 광도는 110 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF로 유지하였다. 두 번째 실험에서는 일장의 영향을 알아보기 위해 순화가 끝난 1개월 묘를 단일 (SD, 06:00~14:00), 중일 (MD, 06:00~1800), 장일 (LD, 06:00~22:00, 야파 (NI, 06:00–18:00 + 22:00–02:00) 조건에서 재배하였다.

저온과 일장의 영향을 알아보기 위해서 각 온도 및 생육단계별로 영양생장과 24시간 동안의 CO_2 흡수율을 측정하였다. 호접란의 광합성은 CO_2흡수 양상에 따라 Phase I부터 IV까지 나뉘어지는 전형적인 CAM 광합성을 보였으며, 3개월간 저온 처리시 하루 동안 흡수한 총 CO_2의 양과 영양생장의 증가 정도가 감소하였다. 특히 3개월 묘를 LT에서 3개월간 재배하였을 때, HS, WR 두 품종의 Phase III동안 CO_2흡수율은 각각 -1.36, -0.60 $\mu mol \cdot m^{-2} \cdot s^{-1}$까지 감소하여 CO_2 가 외부로 누출되는 (CO_2 leakage) 양상을 보였다. 이로 인해 낮 동안 광합성에 사용된 CO_2가 감소하였고, 영양생장량 역시 감소하였다. 1개월 묘를 LT조건에서 재배하였을 때는 신엽의 발생이 통계적으로 유의성있게 감소하였지만, 5개월 묘의 경우에는 감소하지 않았고, 단지 엽장의 생장 정도만 감소하였다. 중일 조건에서 재배하였을 때에는 장일, 단일 조건에서 재배하였을 때보다 엽장, 엽폭, 엽면적이 가장 많이 증가하였다.

본 실험의 결과 어린 묘에서는 저온에 의해 광합성과 영양생장이 더욱 낮게 유지된 것으로 관찰되었으나, 5개월 묘에서는 저온의 영향이 가장 작아 영양생장이 상대적으로 높게 유지됨을 관찰할 수 있었다. 또한 일장의 효과에 있어서는 중일 조건에서 영양생장이 가장 활발하였다는 것을 확인하였다. 따라서 어린 묘를 재배할 때에는 21℃이하의 저온에 노출되지 않도록 하고, 12시간의 일장을 유지하도록 관리하는 것이 필요하다. 이러한 결과는 호접란을 생산하는 과정 중 조직 배양 이후에 어린 묘를 재배, 관리하는데 있어 저온에 의한 피해를 감소하고 양질의 호접란을 생산 할 수 있도록 재배 방법을 개선하는 데에 도움이 될 것이다.